Analysis of selected foods for D-Tagatose

MD FOODS INGREDIENTS AMBA

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Objective.

The objective of this work is to describe a methodology for analysis of D-Tagatose in some common foods. Methodology and data is used to provide support for D-Tagatose as a Generally Recognized As Safe (GRAS) material.

Introduction.

A specific, precise and accurate method has to be available to quantify the levels of D-Tagatose in relevant foods. In this work HPLC is used as the general analytical procedure for the determination of D-Tagatose.

A standard curve is made from different solutions of D-Tagatose. Concentration of added D-Tagatose is plotted against measured peak area, and detection limits is determed.

D-Tagatose has been tested in several foods. Selected test foods containing D-Tagatose are analysed to quantitate the amount of D-Tagatose. The stated amounts of used D-Tagatose are taken from production recipes, and is therefore not exact from an analytical point of view.

The method is validated by spiking/recovery studies, where a certain amount of D-Tagatose is added to blanks without D-Tagatose and afterwards extracted according to the standard procedure. The recovery is calculated. Blanks without added tagatose are analysed to determine possible background signals that could interfere with the signal from D-Tagatose.

The following foods is analysed - chocolate, cereals, soft drinks and ice-creme.

Materials and Methods.

D-Tagatose (crystalline, > 99% purity, > 99.9 dm)

Cereals: (Frosties, Crunchy, Bran Flakes and Corn Flakes) from Kellog®

Soft drinks: (Cola) from Coca-Cola® and test sample from MD Foods Ingredients

Chocolate: Milk chocolate from Toms® and dark chocolate from MD Foods Ingredients

Ice: Ice creme and sherbet ice from Frisko® and ice creme from MD Foods Ingredients

Standard curve.

Solutions of D-Tagatose in the range of 2, 4, 6, 8, 10, 20, 40, 60, 80, 100, 200 and 300 mg/ml was analysed on HPLC, and a standard curve in the range 0-10 mg/ml was plotted with concentration against measured area.

Standard Procedure

Treatment of sample in principle according to Nordic Committee on Food Analysis. No. 155, 1996. (UDC 547.45:577.15:543.9), point 5.1.1 (copy enclosed).
The food is homogenized with a mixer and 5.0 g is dissolved into a 200 ml volumetric flask. Add about 140 ml of water and incubate for 15 minutes at 70 °C in a water bath. The sample is stirred continuously. Allow the sample to cool to room temperature and dilute to 200 ml with water. Place the flask in a refrigerator for 20 minutes to separate fat. The sample is hereafter filtered. Discharge the first few millilitres of the filtrate. The filtered sample is analysed by HPLC.

Soft drinks are analysed directly by HPLC

In the spiking experiments D-Tagatose is added simultaneously with the food sample.

Determination of D-Tagatose by HPLC

Instrumentation

HPLC equipped with a refractive index detector.

column:	Biorad Aminex carbohydrate HPX-87C column (300 mm x 7.8 mm, 9 µm) heated to 85 °C.
mobile phase:	Deionized water with 50 ppm calcium acetate
flow rate:	0.6 ml/min
detector:	Refractive index

Retention time for D-Tagatose under these conditions is app. 16.8 min.

Validation.

The validation studies consists of spiking/recovery studies.

Spiking. Samples of foods are added D-Tagatose at levels of one-half of, equal to and twice the typical use level of D-Tagatose in the actual food.

Recovery. The percent recovery is calculated as $(\frac{a-b}{c}) * 100$

where

"a" is the level of spiked sample analytically determined in the spiked sample

"b" is the background level

"c" is the amount of D-Tagatose added to the sample

Results and Comments.

Standard curve.
Different solutions of D-Tagatose ranging from 0-300 mg/ml was analysed by HPLC. Measured area was plotted against concentration of D-Tagatose. Calibration curve for 0-10 mg/ml is shown in enclosure page no. 6.

R Squared was calculated to be 0.999 showing linearity between 0.2 and 300 mg/ml D-Tagatose. Expected doses of D-Tagatose in foods are inside the detection limits.

Table 1 is showing the results of the spiking experiments and detection of background signals from different foods without D-Tagatose. Only Cola-light gave a minor background signal with app. the same retention time as D-Tagatose.

The spike recovery is calculated as the percentage re-found D-Tagatose in relation to the added amount correlated for background signals. All experiments are done in triple and the standard deviation is calculated.

Table 1. Determination of background signals and spiking recovery.
Samples of food without D-Tagatose are treated according to the standard procedure and analysed by HPLC. Signals with elution time equal to D-Tagatose is called background signal.

Food spiked with D-Tagatose (w/w%)	Background signal "b"	Spike Recovery (%)	SD
Cereals:			
Corn Flakes			
0.0	ntd		
2.5		99.3	7.6
10.0		96.3	4.7
20.0		99.2	1.4
Soft drink:			
Cola			
0.0	ntd		
Cola (light)			

[redacted]

0.0	< 1.5%		
0.1		102.6	7.6
1.0		100.1	4.9
2.0		97.9	6.7
Chocolate:			
Milky (Toms)			
0.0	ntd		
5.0		98.7	3.2
25.0		99.0	2.6
50.0		104.0	3.6
Ice:			
Creme (regnbue)			
0.0	ntd		
5.0		98.1	2.7
10.0		98.0	8.5
20.0		97.6	5.3
Sherbet (filur)			
0.0	ntd		
5.0		85.4	3.1
10.0		87.0	0.8
20.0		87.1	2.2

ntd: no trace detectable
SD: standard deviation

Table 2 is showing the comparison of the added amount of D-Tagatose with the detected amount. The added amounts of D-Tagatose according to the recipes are not exact from an analytical point of view, and minor deviation can be expected.

Examples of HPLC chromatograms for respectively background signals and products with D-Tagatose are shown at page no. 7 and 8.

Table 2. Analysis of D-Tagatose in selected foods.
Samples of foods produced with D-Tagatose are treated according to the standard procedure and analysed by HPLC.

Food	Recipe D-Tagatose (w/w %)	Found D-Tagatose (w/w %)	SD
Cereals:			
Bran flakes	17-19	19.4	0.6
Frosties	36-38	36.1	0.8
Crunchy Nut	25-26	27.9	0.3

Chocolate:			
Dark	25	25.5	0.7
Soft drink:			
Orange	6.7	6.7	0.1
Ice creme:			
Soft Ice	6	6.1	0.1

<u>Enclosure</u> <u>page</u>

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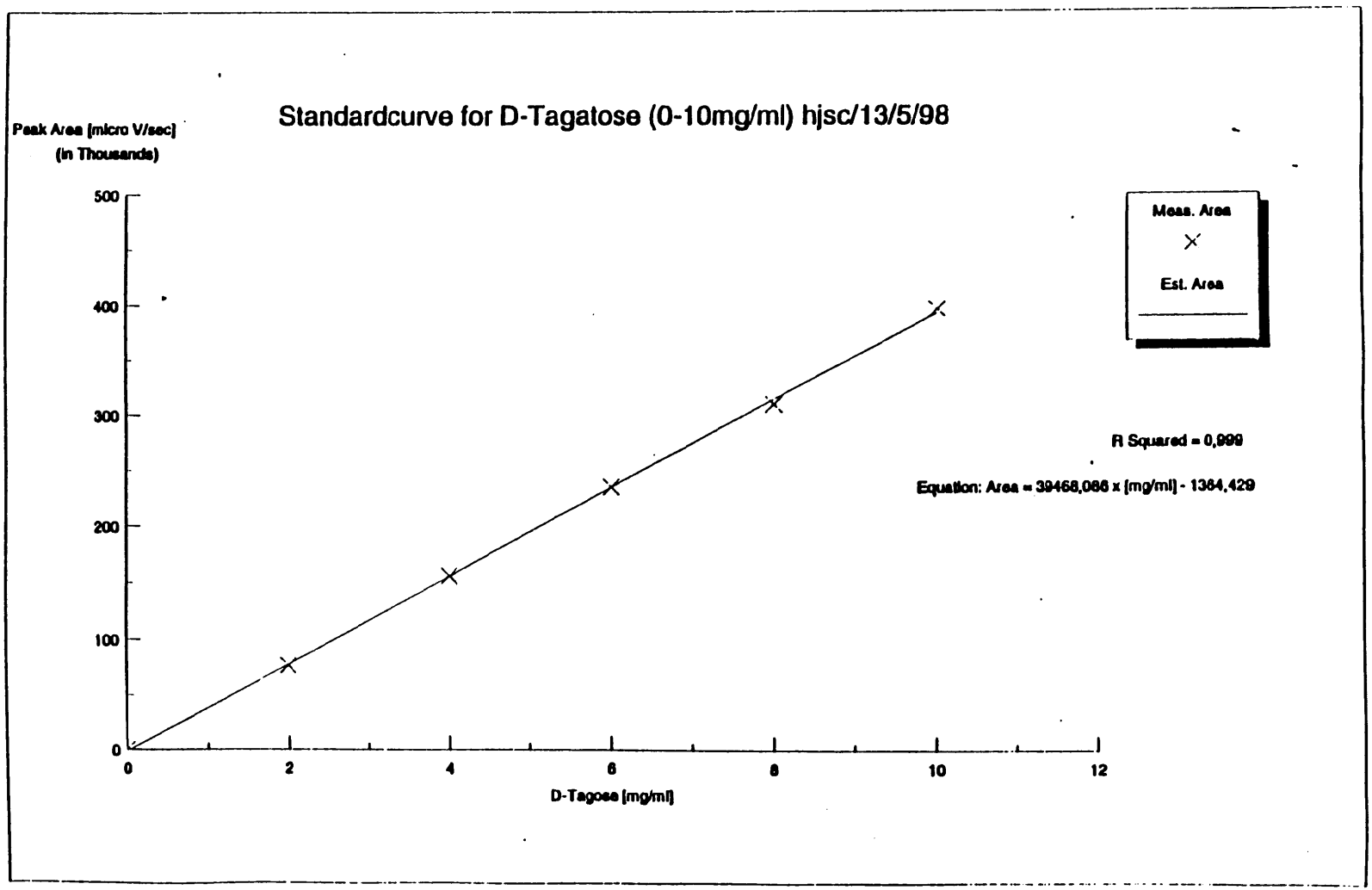
Standardcurve for D-Tagatose (0-10mg/ml) hjsc/13/5/98
Peak Area [micro V/sec]
(in Thousands)
500
400
300
200
100
0
0
2
4
6
8
10
12
D-Tagose [mg/ml]
Meas. Area
Est. Area
R Squared = 0,999
Equation: Area = 39468,086 x [mg/ml] - 1364,429

Cereals

Corn flakes®
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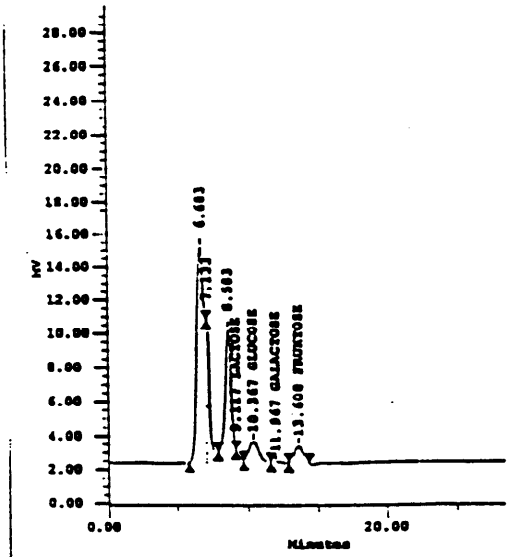
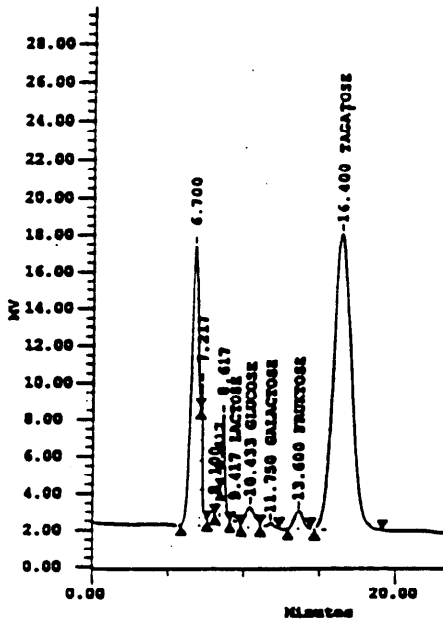


Frosties®
(36-38% w/w D-Tagatose)



Chocolate

Toms®
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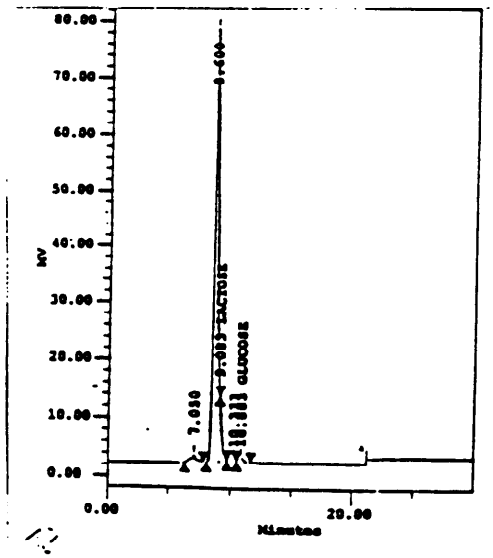
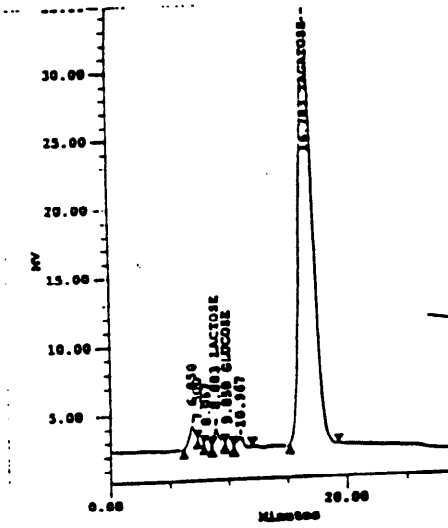


MD Foods
(50% w/w D-Tagatose)



h:\ushvdp102\projects\111112\112170glucose1.dwp

Soft drinks

Cola-light®
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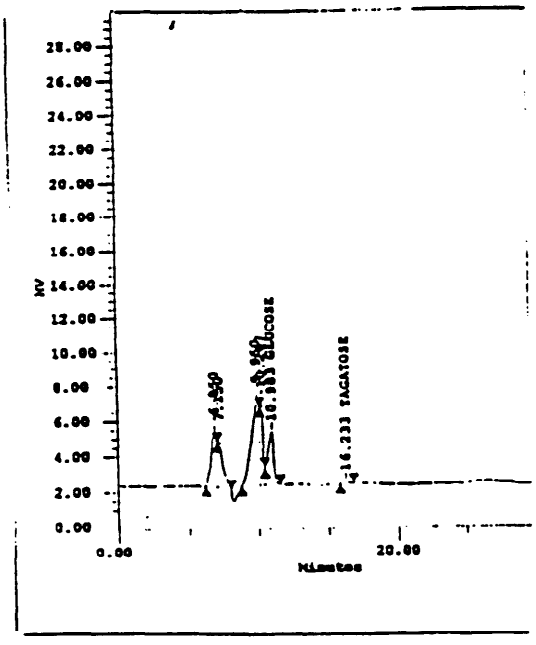
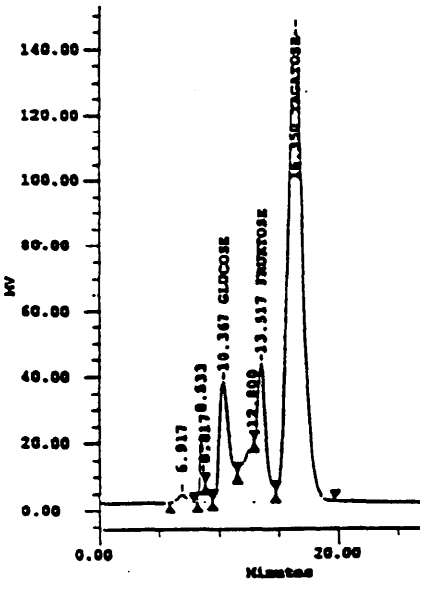


Orange
(6.7% w/w D-Tagatose)



Ice Creme

Frisko®
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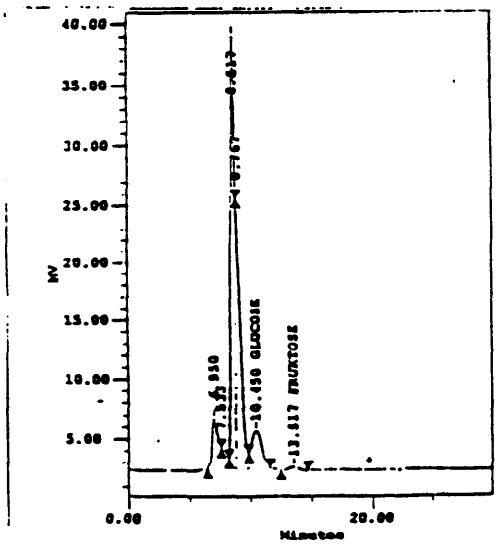

MD Foods
(12% w/w D-Tagatose)